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Docket No. CHL-T104XC1  
Serial No. 09/807,742In the claims:

1 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a biopolymerproinsulin fusion gene, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

2 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastic, a selectable marker sequence, a heterologous DNA sequence coding for a cholera toxin B-subunit-proinsulin fusion gene, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

3 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a plastid DNA fragment comprising a 5'UTR sequence positioned upstream of the promoter to enhance translation of proinsulin protein, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence

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inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

4 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence further coding for a plastid selectable marker sequence to further enhance translation of its proinsulin protein, a transcription termination region functional in said plastid, and flanking each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

5 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a Cry2aA2 operon which comprises two open reading frames (ORF1 and ORF2), wherein the ORF immediately upstream of the Cry2aA2 codes for a putative chaperonin, which assist the crystallization of the insulin and aid in subsequent purification, and which operon is fused directly upstream of the promoter fusion protein, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

6 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a

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selectable marker sequence, a heterologous DNA sequence further coding for a cholera toxin B-subunit-plastid modified proinsulin (PtPris) fusion wherein its nucleotide sequence modified such that the codons are optimized for plastid expression, while its amino acid sequence remains identical to native human proinsulin, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

7 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence further coding for cholera toxin B-subunit-mini-proinsulin (Mpris) fusion wherein its codons are optimized for plastid expression, while its amino acid sequence remains identical to native human proinsulin, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

8 (currently amended). ~~A~~ The vector of claim 1, wherein the biopolymer is a 40mer to enable hyperexpression of the insulin and to accomplish rapid one stop purification of the fusion protein.

9 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence further coding for synthetic protein-base polymer (PBP) fused to a biologically active molecule, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which

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are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

10 (original). A vector of claim 9 wherein the PBP has repeating pentamer sequences (GVGVP)<sub>n</sub>, (SEQ ID NO. 1) wherein "n" is an integer of 1 to 250, "G" is glycine, "V" is valine, and "P" is proline.

11 (original). A vector of claim 9, wherein the biologically active molecule is proinsulin, insulin, or HSA.

12 (original). The vector of claims 1-11, which comprises flanking each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, which sequence is conserved in the plastid genome of different plant species, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target plastid genome.

13 (original). A stable transformed plant which comprises plastid stably transformed with the vector of claims 1-12, or the progeny of the seed thereof.

14 (original). A process for stably transforming a higher target plant species which comprises introducing into the plastid genome of the plant a vector of claims 1-12.

15 (original). A transformed and edible tobacco or alfalfa plant of claim 13.

16 (original). A transformed plant of claim 15 which is edible by humans.

17 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked

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components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for an interferon gene, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

18. (currently amended) A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a an insulin-like growth factor gene, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

19 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a human serum albumin (HSA) gene, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

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20 (original). A stable vector of claim 15 wherein IFN- $\alpha$ 5 is fused to a 5' UTR sequence positioned upstream of the promoter to enhance translation of the IFN- $\alpha$ 5.

21 (original). A stable vector of claim 18 wherein IGF-1 is fused to a 5' UTR sequence positioned upstream of the promoter to enhance translation of the IGF-1.

22 (original). A stable vector of claim 19 wherein HSA is fused to a 5' UTR sequence positioned upstream of the promoter to enhance translation of the HSA.

23 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a cholera toxin B-subunit, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

24 (original). A transformed and edible plant of claim 23.

25 (original). A transformed and edible plant of claim 23, wherein the plant is tobacco or alfalfa.

26 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a biopolymer fusion gene, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding

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sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

27. (currently amended) A process for stably transforming a higher target plant species which comprises introducing into the plastid genome of the plant a vector of claims 9-23[~~7~~] and 26.

28 (original). A process for recovering a biopolymer by a one step extraction and purification by using the reversible property of the biopolymer.

29 (original). A stably transformed plastid of a target plant species of claims 1-12.

30 (original). A process for recovery of a synthetic protein-base polymer (PBP) fused with a biologically active molecule by a one step extraction and purification by using the reversible property of the biopolymer of claim 28.

31 (original). A transformed plastid of a plant of claim 1-12, or the progeny thereof which shows the homoplasmic nature of the transformants.

32 (original). A transformed plastid of a plant of claim 1-12, or the progeny thereof which shows the heteroplasmic nature of the transformants.

33 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for a biopharmaceutical-protein coding gene, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

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34 (original). A stable plastid transformation and expression vector of claim 33, wherein the biopharmaceutical-protein coding gene codes for insulin.

35 (original). A stable plastid transformation and expression vector of claim 34 wherein insulin is natural insulin.

36 (original). A stable plastid transformation and expression vector competent for stably transforming a plastid genome which comprises an expression cassette comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastid, a selectable marker sequence, a heterologous DNA sequence coding for an operon which comprises a putative chaperonin, which assists the crystallization of a protein and aids in subsequent purification, and which operon is fused directly upstream of the promoter fusion protein, a transcription termination region functional in said plastid, and flanking, each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the plastid genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid genome.

37 (original). A stable plastid vector of claim 17 or claim 18, wherein the interferon is alpha 5 IFN- $\alpha$ 5, or the insulin-like growth factor is IGF-1.